Title

Production of 8,8a-dihydroxy-6-deoxyerythronolide B

Cross-Reference to Related Applications

This application claims priority to PCT patent application No. 99/24478, filed 22 Oct. 1999; U.S. patent application Serial No. 09/428,517, filed 28 Oct. 1999; U.S. provisional application Serial Nos. 60/177,660, filed 27 Jan. 00, 60/120,254, filed 16 Feb. 1999, and 60/106,100, filed 29 Oct. 98, each of which is incorporated herein by reference.

Field of the Invention

The present invention provides recombinant methods and materials for producing polyketides by recombinant DNA technology. The invention relates to the fields of agriculture, animal husbandry, chemistry, medicinal chemistry, medicine, molecular biology, pharmacology, and veterinary technology.

Background of the Invention

Oleandomycin (compound (1) of Figure 1) is a member of the macrolide class of antibiotics. Macrolides are a large family of polyketide natural products which include erythromycin, spiramycin, FK506, and avermectin (see Katz *et al.*, *Polyketide synthesis: Prospects for hybrid antibiotics*, *Ann. Rev. Microbiol.* 47: 875-912, 1993; and Hopwood, *Genetic contributions to understanding polyketide synthases*, *Chem. Rev.* 97: 2465-2497, 1997, each of which is incorporated herein by reference). The macrolactone core of oleandomycin, 8,8a-deoxyoleandolide (compound (2) of Figure 1), like those of other macrolides, is synthesized by a modular polyketide synthase (PKS; see Figure 1 and Swan *et al.*, *Characterisation of a Streptomyces antibioticus gene encoding a type I polyketide synthase which has an unusual coding sequence*, *Molec. Gen. Genet.* 242: 358-362, 1994, and U.S. patent application Serial No. 09/428,517, filed 28 Oct. 1999, each of which is

incorporated herein by reference). 8,8a-deoxyoleandolide is structurally identical to the macrolactone precursor of erythromycin, 6-deoxyerythronolide B (6-dEB, see compound (3) of Figure 2), with the exception of a C-13 methyl instead of the C-13 ethyl group of 6-dEB. Thus, 6-dEB is derived from condensations between a propionate starter unit and six methylmalonate extender units, and 8, 8a-deoxyoleandolide has an acetate starter unit.

The study of oleandomycin biosynthesis has been progressive over the past decade, due largely to the identification and sequencing of several biosynthetic and related genes by Salas and coworkers. Analysis of these gene sequences has revealed enzymes putatively involved in synthesis and attachment of the two deoxysugars, regulatory and antibiotic resistance genes, and a P-450 monooxygenase (see Rodriguez *et al., A cytochrome P450-like gene possibly involved in oleandomycin biosynthesis by Streptomyces antibioticus, FEMS Microbiol. Lett.* 127: 117-120, 1995; Olano *et al., Analysis of a Streptomyces antibioticus chromosomal region involved in oleandomycin biosynthesis, which encodes two glycosyltransferases responsible for glycosylation of the macrolactone ring, Mol. Gen. Genet.* 259: 299-308, 1998; and Quiros *et al., Two glycosyltransferases and a glycosidase are involved in oleandomycin modification during its biosynthesis by Streptomyces antibioticus, Mol. Microbiol.* 28: 1177-85, 1998, each of which is incorporated herein by reference). Thus, a single open reading frame (ORF) encoding a polypeptide subunit of a type I polyketide synthase was identified and, based on comparison to 6-deoxyerythronolide B synthase (DEBS), was hypothesized to encode the last two modules of the oleandomycin PKS (OlePKS; see Swan *et al., supra*). The cloning, characterization, and sequence determination of the other genes encoding the proteins of the OlePKS are described in PCT patent publication No. 00/026,349, incorporated herein by reference.

There remains, however, a need for additional methods and reagents to produce 8,8a-deoxyoleandolide, oleandomycin, and related compounds in heterologous host cells. The present invention meets these and other needs.

Summary of the Invention

The gene cluster encoding the deoxyoleandolide polyketide synthase (OlePKS) was isolated from the oleandomycin producing strain *Streptomyces antibioticus*. Sequencing of the first two genes encoding OlePKS, together with the previously identified third gene revealed an overall genetic and protein architecture similar to that of the erythromycin gene cluster encoding the 6-deoxyerthronolide B synthase (DEBS) from *Saccharopolyspora erythraea*. When the entire OlePKS (10,487 amino acids) was expressed in the heterologous host *Streptomyces lividans*, it produced 8,8a-deoxyoleandolide (compound (2) of Figure 1), the aglycone precursor of oleandomycin. The P450 monooxygenase, OleP, involved in oleandomycin biosynthesis was co-expressed with DEBS in *S. lividans*. The production of 8,8a–dihydroxy-6-deoxyerythonolide B (compound (6) of Figure 2) and other derivatives demonstrates that OleP is involved in the epoxidation pathway of oleandomycin biosynthesis. Further, a method of producing 8,8a-dihydroxy-6-deoxyerythonolide B or 8,8a-dihydroxyoleandolide comprising the heterologous expression of DEBS or Ole PKS, respectively, with OleP in *S. lividans* was developed. This heterologous expression system provides a means to produce these compounds, which can directly or after further modification be hydroxylated and glycosylated to provide useful macrolide antibiotics.

Thus, in one embodiment, the present invention provides a method for introducing one or more hydroxyl groups or an epoxide into a polyketide, which method comprises expressing a recombinant gene encoding a P450 monoxygenase in a host cell. In one embodiment, the P450 monooxygenase is not naturally expressed by the host cell. In another embodiment, neither the P450 monooxygenase nor the polyketide is naturally expressed by the host cell. In a preferred embodiment, the host cell is a *Streptomyces* host cell. In another preferred embodiment, the P450 monooxygenase is OleP. In another preferred

embodiment, the polyketide synthase is 6-deoxyerythronolide B synthase or 8,8a-deoxyoleandolide synthase.

Brief Description of the Figures

Figure 1 shows a schematic for the biosynthesis of 8,8a-deoxyoleandolide (compound (2)), the macrolactone precursor of oleandomycin (compound (1)). The oleandomycin PKS consists of six modules, a loading domain, and a thioesterase (TE) on three separate polypeptides. In the Figure, the following abbreviations are used: KS, ketosynthase; AT, acytransferase; KR, ketoreductase; DH, dehydratase, ER, enoylreductase; ACP, acyl carrier protein; KS^Q, a KS-like domain that contains instead of an active-site cysteine a glutamine.

Figure 2 shows a proposed hydroxylation pathway of 6-dEB by OleP in *S. lividans*. This pathway proceeds through the novel intermediate compound of the invention 8,8a-dehydro-6-deoxyerythronolide B (compound 4). If the OlePKS instead of DEBS were employed, then the compound produced is 8,8a-anhydrooleandolide.

Detailed Description of the Invention

The present invention relates to the heterologous expression of the OlePKS coding sequence and/or OleP, the P-450 hydroxylase that converts 8,8a-deoxyoleandolide to oleandolide in *Streptomyces antibioticus*. The OlePKS is encoded by three ORFs - *oleAI*, *oleAII*, and the previously identified ORFB (see Swan *et al., supra*; ORFB is designated *oleAIII*) - that span 35 kb of DNA. Each of the ORFs encodes two PKS modules, as in *eryAI-AIII*, and examination of the active site domains within the modules also reveals an organization similar to the active site arrangement of DEBS, as shown in Table 1, below. The sequence of the OlePKS genes and recombinant vectors from which those genes can be isolated are described in PCT publication No. 00/026349, incorporated herein by reference.

Table 1

Functions of oleandomycin PKS domains

ORF	Amino acids	Deduced function					
OleAI	4151						
Loading	1025	KS^Q	AT	ACP			
Module 1	1465	KS	AT	KR	ACP		
Module 2	1520	KS	AT	KR	ACP		
OleAII	3817						
Module3	1543	KS	AT	KR	ACP		
Module4	2117	KS	AT	DH	ER	KR	ACP
OleAIII	3520						
Module5	1531	KS	AT	KR	ACP		
Module6	1532	KS	AT	KR	ACP	TE	

The similarity observed between the OlePKS and DEBS was not unexpected given the structural relationship of the polyketides produced by the enzymes encoded by the two gene clusters. Yet the amino acid sequences (45% aa identity) of OlePKS and DEBS are surprisingly different from one another. A significant departure was found in the OlePKS loading domain.

In contrast with the loading module of DEBS, which consists of an acyl transferase (AT) domain - that loads a propionate starter unit - and an acyl carrier protein (ACP), the OlePKS loading module has an additional KS-like domain (KS^Q) with a glutamine instead of a cysteine at the active site. These domains have been shown to decarboxylate acylthioesters within PKSs and the related fatty acid synthases (see Witkowski *et al., Conversion of a ß-ketoacyl synthase to a malonyl decarboxylase by replacement of the active-site cysteine with glutamine,* Biochemistry, 1999; and Bisang *et al., A chain initiation factor common to both modular and aromatic polyketide synthases, Nature* 401: 502-505, 1999, each of which is incorporated herein by reference). Therefore, the OlePKS is believed to

initiate 8,8a-deoxyoleandolide synthesis by loading the ACP with a malonate unit and performing a decarboxylation to generate acetyl-ACP.

The heterologous production of 8,8a-deoxyoleandolide can be accomplished in *Streptomyces lividans* and *S. coelicolor*. Preferred strains of these organisms are described in U.S. Patent No. 5,672,491 and U.S. patent application Serial No. 09/181,833, filed 28 Oct. 1998, incorporated herein by reference. A vector for heterologous expression of the OlePKS was constructed in a manner analogous to those developed for DEBS and the picromycin PKS (PicPKS; see Kao *et al., Engineered biosynthesis of a complete macrolactone in a heterologous host, Science* 265: 509-512, 1994; Tang *et al., Elucidating the mechanism of chain termination switching in the picromycin/methymycin polyketide synthase, Chem. & Biol.* 6: 553-558, 1999; and PCT publication No. 99/61599, each of which is incorporated herein by reference). Plasmid pKOS098-4 is an autonomously replicating SCP2*-based shuttle vector containing the three OlePKS open reading frames downstream of the *S. coelicolor actI* promoter and *act*II-ORF4 transcriptional activator.

Transformation of *Streptomyces lividans* K4-155 with this vector resulted in a strain that produced ~50 mg/L of compound (2) of Figure 1, confirming that 8,8a-deoxyoleandolide is the biosynthetic intermediate produced by the oleandomycin PKS. The mass spectrum and LC retention time of compound (2) of Figure 1 were identical to known standards. The amount of 8,8a-deoxyoleandolide produced is similar to production levels of 6-dEB and narbonolide achieved with DEBS and PicPKS, respectively, using the same host and vector (Kao *et al., Science* 265: 509-512, 1994; and Tang *et al., Chem. & Biol.* 6: 553-558, 1999, each of which is incorporated herein by reference).

The invention further provides a method of producing mono and dihydroxy derivatives of 6-deoxyerythronolide B and 8,8a-deoxyoleandolide in recombinant host cells. In one embodiment, the 6-dEB compound is produced by recombinant expression of a 6-dEB synthase, such as that produced by the *eryA* or *meg* genes (the meg genes are described in PCT patent application

US00/27433, incorporated herein by reference). In another embodiment, 8,8a-deoxyoleandolide is produced by recombinant expression of the OlePKS.

For example, 8,8a-dihydroxy-6-deoxyerythonolide B can be produced by the heterologous expression of DEBS with OleP in a *Streptomyces* host cell, such as *S. lividans*. The *oleP* gene is located approximately 6 kb downstream of the end of *oleAIII* (see Rodriguez *et al., A cytochrome P450-like gene possibly involved in oleandomycin biosynthesis by Streptomyces antibioticus., FEMS Microbiol. Lett.* 127: 117-120, 1995, incorporated herein by reference). This gene encodes a cytochrome P450 monooxygenase homologous to several macrolide oxidases such as those found in the erythromycin (Weber *et al., An erythromycin derivative produced by targeted gene disruption in Saccharopolyspora erythraea., Science* 252: 114-117, 1991; Haydock *et al., Cloning and sequence analysis of the genes involved in erythromycin biosynthesis in Saccharopolyspora erythraea; sequence similarities between eryG and a family of S-adenosylmethionine-dependent methyltransferases., Mol. Gen. Genet.* 230: 120-128, 1991; and Stassi *et al., Identification of a Saccharopolyspora erythraea gene required for the final hydroxylation step in erythromycin biosynthesis., J. Bacteriol.* 175: 182-189, 1993, each of which is incorporated herein by reference), picromycin/methymicin (Betlach *et al., Characterization of the macrolide P-450 hydroxylase from Streptomyces venezuelae which converts narbomycin to picromycin., Biochemistry* 37: 14937-14942, 1998; and Xue *et al., Hydroxylation of macrolactones YC-17 and narbomycin is mediated by the pikC-encoded cytochrome P450 in Streptomyces venezuelae., Chem. & Biol.* 5: 661-667, 1998, each of which is incorporated herein by reference), and tylosin gene clusters (Merson-Davies *et al., Analysis of five tylosin biosynthetic genes from the tylIBA region of the Streptomyces fradiae genome., Mol. Microbiol.* 13: 349-355, 1994, incorporated herein by reference).

Although the gene product OleP is putatively involved in formation of the oleandomycin epoxide moiety, several experiments have failed to establish its role (Rodriguez *et al., FEMS Microbiol. Lett.* 127: 117-120, 1995, incorporated

herein by reference). Furthermore, the biochemical mechanism of epoxidation is not known and, therefore, whether any other enzymes are required in addition to OleP is also not known. When the epoxidation step occurs during biosynthesis is also not understood (see Spagnoli *et al., Biological conversion of erythronolide B, an intermediate of erythromycin biogenisis, into new "hybrid" macrolide antibiotics, J. Antibiotics* 36: 365-375, 1983, and Tatsuta *et al., Biosynthetic studies on oleandomycin by incorporation of the chemically synthesized aglycones, J. Antibiotics* 43: 909-911, 1990, each of which is incorporated herein by reference).

The present invention resolves many of these uncertainties and further provides methods for producing novel polyketides by the heterologous expression of OleP and a PKS such as DEBS or the OlePKS. In one illustrative embodiment, the plasmid pKAO127'kan' (see Ziermann, R. *et al., Recombinant polyketide synthesis in Streptomyces: Engineering of improved host strains, Biotechniques* 26: 106-110, 1999, incorporated herein by reference) a DEBS expression plasmid that produces both 6-dEB (Figure 2, compound (3)) and 8,8a-deoxyoleandolide (Figure 1, compound (2)) in *Streptomyces lividans,* was used for coexpression with OleP.

The *oleP* gene was integrated into the chromosome of *S. lividans* K4-114/pKAO127'kan' using a phiC31-based vector under control of the *actII*-ORF4 activator and P*act*I elements. The resulting strain produced a mixture of compounds, of which the predominant components were 8,8a-deoxyoleandolide (compound (2) of Figure 1) and 6-dEB (~1:7) (compound (3) of Figure 2). At least six additional compounds were present at levels between 10-50% of 8,8a-deoxyoleandolide, and all appear to be derivatives of 6-dEB, based on MS analysis. Collectively, this amounts to ~33% conversion of the 6-dEB produced by the strain. There were no derivatives of 8,8a-deoxyoleandolide detected, presumably because it was produced in such low quantity compared to 6-dEB in this analysis. However, if the OlePKS were expressed instead of DEBS, then the compounds produced would include 8,8a-dihdroxyoleandolide.

Of the compounds identified, two appeared to be singly hydroxylated species, three appeared to be dehydro derivatives, and one compound appeared to be a dihydroxylated compound when mass spectra were compared to that of 6-dEB. The dihydroxy derivative was selected for structural validation. The structure of this compound 8,8a-dihydroxy-6-deoxyerythonolide B (compound (6) of Figure 2), was established using mass spectrometry and NMR spectroscopy.

HRFABMS was obtained for the $[M\text{-}H_2O+H]^+$ ion ($C_{21}H_{37}O_7$, calculated: 401.2539; observed: 401.2534). DEPT spectra indicated the presence of six methyl, three methylene, nine methine, and three quaternary carbons, consistent with the proposal that a methyl and methine from 6-DEB had been replaced by an oxy-methylene (delta 67.7, C-8a) and oxy-quaternary carbon (delta 91.6, C-8). HMQC NMR data allowed the assignment of two protons resonating at delta 3.45 (d, 11.5 Hz) and 3.60 (d, 11.5 Hz) to C-8a. HMBC correlations between H_a-8a and C-8 as well as carbons signals at delta 40.8 (C-7) and 219.1 (C-9) confirmed that the oxidations had occurred at the 8 and 8a positions. Additional NMR data from TOCSY, HMQC, and HMBC (Table 2, below) experiments fully support the assigned structure of 8,8a-dihydroxy-6-deoxyerythonolide B. The absolute stereochemical configuration at C-8 has not been determined.

Table 2

^{1}H and ^{13}C NMR data for 8,8a-dihydroxy-6-deoxyerythonolide B[a]

position	^{1}H delta pm (m, *J*HH)	^{13}C delta ppm (m)	HMBC correlations
1		176.2 (s)	
2	2.66 (dq, 10.5, 6.5)	45.4 (d)	C-1, C-2a, C-3, C-4
2a	1.23 (d, 6.5)	13.7 (q)	C-1, C-2, C-3
3	3.80 (d, 10.5)	72.9 (d)	C-1, C-2, C-2a, C-4, C4a, C-5
4	1.93 (m)	42.8 (d)	C-2, C4a, C-5
4a	1.19 (d, 7.0)	12.0 (q)	C-5
5	3.50 (ovrlp)	90.8 (d)	
6	1.73 (ovrlp)	36.5 (d)	
6a	1.07 (d, 6.0)	17.6 (q)	C-6, C-7, C-8
7	1.56 (dd, 12.5, 12.5)	40.8 (t)	C-8
	2.57 (dd, 12.5, 7.0)		C-5
8		91.6 (s)	
8a	3.45 (d, 11.5)	67.7 (t)	C-7, C-8, C-9
	3.60 (d, 11.5)		C-9
9		219.1 (s)	
10	3.50 (ovrlp)	41.2 (d)	
10a	0.95 (d, 7.5)	7.4 (q)	
11	3.59 (m)	69.2 (d)	
12	1.71 (m)	41.6 (d)	
12a	0.93 (d, 7.5)	9.0 (q)	C-11, C-12
13	5.36 (ddd, 9.5, 7.0, <1)	76.2 (d)	C-1, C-11, C-12, C12a, C-14, C15
14	1.51 (m)	25.7 (t)	
	1.74 (m)		
15	0.90 (t, 7.5)	10.4 (q)	C-13, C-14

a. Recorded in $CDCl_3$

Based on the structure of 8,8a-dihydroxy-6-deoxyerythonolide B, one can conclude that OleP is partly or wholly responsible for introduction of the oleandomycin epoxide and that this step most likely occurs prior to deoxysugar attachment. The presence of the diol could be explained by formation of the epoxide (compound (5) of Figure 2) and subsequent hydrolysis by an endogenous enzyme in *Streptomyces lividans* to 8,8a-dihydroxy-6-

deoxyerythonolide B. This is further supported by the presence of small amounts of a dehydro derivative (putatively at carbons C-8,8a) compound (compound (4) of Figure 2) in the mixture. An alternative explanation is that an additional enzyme(s) present in *S. antibioticus* is required for formation of the epoxide, and in its absence, OleP performs the double hydroxylation. In either case, this result suggests that epoxide formation occurs prior to attachment of the two sugars, which was heretofore unknown.

The following examples are given for the purpose of illustrating the present invention and shall not be construed as being a limitation on the scope of the invention or claims.

Example 1

Bacterial strains and culture conditions

DNA manipulations were performed in *Escherichia coli* XL1-Blue (Stratagene) and DH10B (BRL). *Streptomyces lividans* K4-114 and K4-155, genotypically identical strains which contain deletions of the entire actinorhodin gene cluster, were used as host strains for the production of polyketide compounds (see Ziermann *et al., Recombinant polyketide synthesis in Streptomyces: Engineering of improved host strains, Biotechniques* 26: 106-110, 1999, and U.S. patent application Serial No. 09/181,833, filed 28 Oct. 1998, each of which is incorporated herein by reference). *S. lividans* was transformed according to standard methods, and clones were selected with thiostrepton (50 μg/ml) or apramycin (200 μg/ml) overlays on R5 regeneration plates (see Hopwood *et al., Genetic Manipulation of Streptomyces: A Laboratory Manual, The John Innes Foundation,* Norwich, U.K., 1985, incorporated herein by reference).

Example 2

Cloning of the oleandomycin biosynthesis gene cluster from *Streptomyces antibioticus*

Genomic DNA was isolated from an oleandomycin producing strain of *S. antiobioticus* (ATCC 11891) using standard procedures (see Hopwood *et al.*, *supra*). A genomic library was prepared in Supercos™ (Stratagene) using DNA partially digested with *Sau*3A I following the supplier's protocols. A probe was prepared by PCR amplification of genomic DNA using primers specific to the KS domains of modules 5 and 6 of OlePKS. The genomic library was then probed by colony hybridization with ^{32}P-labeled probe. Cosmids containing the desired DNA inserts were verified by PCR with the same primers and by comparison of restriction digest patterns to known sequences. Two overlapping cosmids, pKOS055-5 and pKOS055-1, were identified which cover approximately 65 kb of DNA and contain the entire oleandomycin gene cluster. See PCT publication No. 00/026349 and U.S. patent application Serial No. 09/428,517, filed 28 Oct. 1999, each of which is incorporated herein by reference.

Example 3

DNA sequencing and analysis

Six fragments ~5 kb in size and containing the desired region of the PKS to be sequenced were subcloned from cosmid pKOS055-5. Shotgun libraries were made from each subclone using *Hin*P1 I partially digested DNA cloned into pUC19. Insert sizes ranged from 500-3000 bp. PCR-based double-stranded DNA sequencing was performed on the shotgun clones using a Beckman CEQ 2000 capillary sequencer. Modules 1-4 of the PKS gene cluster were sequenced to approximately 4x coverage. Sequence was assembled using the Sequencher™ (Gene Codes Corp.) software package and analyzed with MacVector (Oxford Molecular).

Example 4

Construction of expression plasmids for OlePKS and OleP

The OlePKS expression plasmid pKOS098-4 was constructed by replacing the *eryAI-AIII* genes between the *Nde* I and *Eco*RI sites of pKAO127'kan' (Ziermann *et al., supra*) with the *oleAI-AIII* genes. A 15.2-kb *Nsi* I-*Eco*R I fragment containing *oleAI* and a portion of *oleAII* from cosmid pKOS055-5 was subcloned into a vector containing an *Nde* I site 3 nucleotides (nt) from the 5' terminus of the *Nsi* I site to generate pKOS039-116. The 15.2-kb *Nde* I-*Eco*R I fragment was then subcloned into another vector containing a *Pac*I site 15 nt from the 5' terminus of the *Nde* I site resulting in pKOS039-110. This generated the following sequence upstream of the *Nsi* I site in *OleAI* (*Pac* I and *Nsi* I sites are underlined, *Nde* I site is in bold): 5'-TTAATTAAGGAGGACC**ATATG**CAT-3'. The 15.2 kb *Pac* I-*Eco*R I fragment from pKOS039-110 was then cloned into the corresponding sites of pKAO127'kan' to yield pKOS038-174.

Next, a 14-kb *Eco*RI-*Eco*RV fragment and a 5.4-kb *Eco*RV-*Pst* I fragment, together containing the remaining portions of the *oleAII* and *oleAIII* genes, were obtained from cosmid pKOS055-1 and cloned concomitantly into pLitmus28 (Stratagene) to give pKOS039-115. A 20-kb *Spe*I-*Xba* I encompassing both of the former fragments was then excised and subcloned into another vector to introduce an *Eco*RI cloning site downstream of the *oleAIII* gene. This allowed a 20-kb *Eco*RI fragment to be extracted from this plasmid and inserted into the *Eco*R I site of pKOS038-174 (see above) to complete construction of the OlePKS expression vector pKOS098-4.

The *ole*P gene was PCR amplified using the following oligonucleotide primers (forward, 5'-TTTCATATGGTGACCGATACGCACACCGGA-3', reverse, 5'-TTTGAATTCTCACCAGGAGACGATCTGGCG-3'). After subcloning in PCRScript (Stratagene), the *Nde* I-*Eco*RI fragment containing *oleP* was isolated and cloned into pSET152-based plasmid pKOS010-153 (see Xue *et al., A multi-plasmid approach to preparing large libraries of polyketides, Proc. Natl. Acad. Sci.* USA

96: 11740-11745, 1999, incorporated herein by reference) replacing the *Nde* I-*Eco*R I *eryAIII* gene fragment to yield pKOS024-83.

Example 5

Production and analysis of polyketide analogs

Streptomyces lividans transformants were cultured in 5 ml of liquid R5 medium (see Hopwood *et al., supra*) at 30°C for 7 days under the appropriate antibiotic selection (thiostrepton 6 μg/ml, apramycin 50 μg/ml). Samples of fermentation broth were analyzed as previously described by LC/MS on a reverse-phase C-18 column (4.6 x 150 mm) using a Perkin-Elmer SCIEX API100 mass spectrometer. The polyketides were identified by correspondence of their mass spectrum to the products expected or to known standards. Compound 8,8a-dihydroxy-6-deoxyerythonolide B was purified by silica gel chromatography using ethyl acetate/hexane (1:1). Fractions were analyzed by mass spectrometry and those containing the desired compound were pooled together. The structure of 8,8a-dihydroxy-6-deoxyerythonolide B was determined by NMR and mass spectroscopy (details in Table 2, above).

The invention having now been described by way of written description and examples, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples are for purposes of illustration and not limitation of the following claims.